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Japanese Published Unexamined (Kokai) Patent Publication No. S60-47692; Publication Date: March 15, 1985; Application No. S58-155333; Application Date: August 25, 1983; Int. Cl.⁴: C12P 13/08 // (C12P 13/08 C12R 1:185); Inventor(s): Takayasu Tsuchida et al.; Applicant: Ajinomoto Corporation; Japanese Title: Hakkouhou niyoru L-Sureonin no Seizouhou (Method for Production of L-Threonine by a Fermentation Means)

Specification

1. Title of Invention

Method for Production of L-Threonine by a Fermentation Means

2. Claim

A method for production of L-threonine by a fermentation means, characterized in that a microorganism inhering an Escherichia family L-threonine producing capability is cultivated in a liquid culture containing lactose or galactose as a main carbon source to extract L-threonine generated and deposited in the culture.

3. Detailed Description of the Invention

This invention pertains to a producing method for L-threonine by a fermentation means.

L-threonine is produced from carbon sources such as glucose, sucrose, acetic acid and the like using coryne bacteria of the Bureibacterium family and the Corynebacterium family.

Nevertheless, these conventional coryne bacteria cannot materialize both lactose and galactose. Accordingly, L-threonine cannot be produced using economical raw materials that contain these sugars, such as milk whey.

In relation to the conventional L-threonine producing method as described above, the inventors have succeeded to grow strains inhering an ability to generate L-threonine from the Escherichia family at a high yield using lactose or galactose as a carbon source, thereby attaining the invention.

The following are organisms that inhere a producing capability for Escherichia L-threonine: Escherichia-Cory AJ 11332 (FERM-P4878); Escherichia-Cory AJ 11334 (FERM-P4900); Escherichia-Cory AJ 11335 (FERM-4901).

Escherichia-Cory AJ 11332 (FERM-P4878) is already known as a variant having resistance to α -amino- β -hydroxy valeric acid (henceforth referred to as AHU) (as disclosed in Japanese examined patent application S45-26709) and obtained by applying a regular artificial variation operation to the Escherichia microorganisms. On the other hand, Escherichia-Cory AJ 11334 (FERM-P4900) and Escherichia-Cory AJ 11335 (FERM-4901) are already known as L-threonine producing strains that contain plasmid in which deoxyribonucleic acid carrying genetic information related to biosynthesis of L-threonine obtained from the variant having resistance to AHU is incorporated (Japanese examined patent application No. S55-131397) and obtained by a DNA means.

The growing process of Escherichia-Cory AJ 11332 (FERM-P4878), Escherichia-Cory AJ 11334 (FERM-P4900) and Escherichia-Cory AJ 11335 (FERM-4901) is disclosed in Japanese unexamined patent application No. S55-131397.

The culture used for cultivating the microorganisms inhering the producing capability for the Escherichia L-threonine as described above is not as a particular type except that it contains lactose or galactose as a carbon source. As for lactose and galactose, milk whey, soybean whey and the like can also be used. Other than these carbon sources, xylose, glucose, sucrose, maltose and the like are sometimes contained in the culture as sub-carbon sources. Regular nitrogen sources such as ammonium ions, ammonia gas and ammonia water, inorganic ions such as phosphoric ions, magnesium ions and potassium ions and if necessary organic nutrients such as vitamins and amino acid are contained in the culture.

The cultivation is applied under aerobic conditions. During the cultivation, the temperature is preferably maintained at a proper temperature in the range between 27°C and 37°C whereas the pH of the culture is maintained at a proper pH in the range between 5.0 and 8.0. Accordingly, when the cultivation is continued for 1 to 4 days, a significant amount of L-threonine is generated and deposited in the culture.

A regular method is used to extract L-threonine deposited on the culture.

Working Example 1

A carbon source at 3%, $(\text{NH}_4)_2\text{SO}_4$ at 1%, KH_2PO_4 at 0.2%, $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ at 0.1%, Fe^{++} at 2 ppm, Mn^{++} at 2 ppm, thiamine hydrochloride at 1 mg/l, L-proline at 300 mg/l, L-isoleucine at 100 mg/l, L-methionine at 100 mg/l and CaCO_3 at 2% (adjusted to pH 7.0 using KOH) are supplied in a Sakaguchi's flask at 20 ml each. After various types of strains have been inoculated, Cultivation by a lid oscillation is applied at 37°C for 70

hours. As indicated in Table 1, L-threonine is deposited at a yield higher than that of lactose and galactose.

Table 1

Carbon sources	Strains [Please refer to the original description]	L-threonine (g/l)
Lactose		
Galactose		
Added in the form of milk whey lactose		

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